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Effect of dietary long-chain fatty acids on the rates of cholesterol turnover processes, cholesterol origin and distribution in the rat intestinal lumen

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Summary. Adult male rats were fed a semi-purified diet contaning 20 p. 100 of lard or homogeneous triglycerides (tripalmitin, tristearin, triolein, trierucin) for 4 months. The rates of the processes (absorption, synthesis, degradation into bile acids, fecal and urinary excretion) involved in cholesterol turnover were measured using the isotope equilibrium method.

The absorption coefficient of dietary cholesterol attained 71 $\pm$ 3 p. 100 in rats fed the lard diet. It was unchanged in rats eating the triolein (73 $\pm$ 3 p. 100) or tripalmitin (75 $\pm$ 4 p. 100) diet but was drastically reduced in those receiving trierucin (45 $\pm$ 1 p. 100) or tristearin (34 $\pm$ 1 p. 100). Some functional and topological aspects of cholesterol absorption are discussed. Four to 6 times more endogenous cholesterol was found in the intestinal lumen of rats fed trierucin than in that of rats receiving a normolipid diet. Micelle cholesterol increase was also proportionally less. There was no direct relationship between the quantity of cholesterol found in the micelles and that absorbed. Dietary cholesterol was absorbed in the second as well as in the first half of the intestine, while the proportions of micellar cholesterol decreased continuously from the jejunum to the ileum.

The rates of fecal excretion and fecal external secretion were inversely correlated to the absorption coefficient of dietary cholesterol, as has been previously described. The internal secretion was stimulated two to threefold in rats fed tristearin (22.3 $\pm$ 0.7 mg/d), tripalmitin (28.4 $\pm$ 4.6 mg/d) and trierucin (33.2 $\pm$ 2.8 mg/d) as compared to rats fed lard (12.5 $\pm$ 0.6 mg/d) or triolein (14.4 $\pm$ 1.2 mg/d). Feeding a tripalmitin or a trierucin diet greatly increased cholesterogenesis (probably in the liver), while tristearin stimulated non-hepatic cholesterogenesis. The variations in daily bile acid elimination may be seen as an adaptive parameter, modulating its rate according to the cholesterol supply (absorption and synthesis).

Introduction.

Numerous studies have shown that the nature of dietary fatty acids affects the rates of the cholesterol turnover processes (for review see McGandy and Hegsted, 1975 ; Grundy, 1976 ; Truswell, 1977 ; Chevallier, 1977), but the data are generally difficult to interpret because of the complex nature of the ingested lipid (natural oil,

for example) or because only one cholesterol turnover process was studied. The isotope equilibrium method, however, permits *in vivo* measurement of the turnover rates of mobile cholesterol, i.e. absorption, internal secretion, urinary and fecal excretion and transformation into bile acids (Chevallier and Lutton, 1966 ; Chevallier, 1967 ; Lutton and Chevallier, 1972*a*), and of some parameters (external secretion, for example) which concern cholesterol elimination via the feces (Chevallier and Lutton, 1966 ; Chevallier, 1967 ; Lutton, 1976). In the present study, this method has been applied to rats fed a semi-purified diet containing 20 p. 100 of homogeneous triglycerides (tripalmitin, tristearin, triolein or trierucin) or 20 p. 100 of lard. Since there were large changes in some of the rates of the cholesterol turnover processes, particularly absorption, the origin of cholesterol and some of its topological aspects in the intestinal contents have also been studied.

Experimental procedure.

Material. — 4-^{14}C-cholesterol and ^{3}H-G-cholesterol (40-50 mCi/mmole) were purchased from the CEA (France). Their purity, checked by thin-layer chromatography on Silica Gel-G with hexane/ethylacetate (80 : 20, v/v), was found to be $\geqslant$ 98 p. 100 (Sulpice *et al.*, 1978). Triolein was purchased from Prolabo (France) and tripalmitin and tristearin from Fluka AG ; the trierucin was a gift from Astra-Calvé. The purity of these three triglycerides was $\geqslant$ 99 p. 100.

TABLE 1

Composition of experimental diets

	U g/100 g	Ol g/100 g	Pa g/100 g	St g/100 g	Er g/100 g
Saccharose	54	54	54	54	54
Casein	10	10	10	10	10
L cystine	0.2	0.2	0.2	0.2	0.2
Lipids					
Oleate/linoleate (1/1)	2	2	2	2	2
Lard	20	—	—	—	—
Triolein	—	20	—	—	—
Tripalmitin	—	—	20	—	—
Tristearin	—	—	—	20	—
Trierucin	—	—	—	—	20
Skim-milk	4	4	4	4	4
Agar-agar	2.3	2.3	2.3	2.3	2.3
Vitamins (1)	0.2	0.2	0.2	0.2	0.2
Yeast	2.3	2.3	2.3	2.3	2.3
Salt mixture (2)	5	5	5	5	5

(1) Vitamins : Vitamins (mg/100 g diet) : retinyl acetate, 1 350 IU ; ergocalciferol, 100 IU ; DL-α-tocopherol 50 ; biotin, 0.1 ; vitamin B_{12}, 0.002 ; thiamin, 2 ; riboflavin, 2 ; pyridoxine, 2 ; nicotinic acid, 9 ; folic acid, 0.2 ; calcium pantothenate, 5 ; menadione, 4.5 ; meso-inositol, 85 ; choline, 100 ; vitamin C, 90.

(2) Salt mixture (mg/100 g diet) : NaCl, 456 ; KH_2PO_4, 1 388 ; $Ca(PO_4H_2)_2$, H_2O, 1 388 ; $MgSO_4$, 771 ; NaH_2PO_4, 601 ; $Ca(CH_3CHOHCOO)_2$, 5 H_2O, 288 ; $Fe(CH_3H_5O_2)_2$, 3 H_2O, 118 ; NaF, 0.15 ; $MnSO_4$, H_2O, 0.37 ; $Al_2(SO_4)_3K_2SO_4$, 24 H_2O, 0.5 ; KI, 3.25 ; $ZnCl_2$, 10.4 ; $CoCl_2$, 6 H_2O, 0.3.

Animals and diets. — After weaning, male Wistar rats were fed a basal, semi-purified diet (Chevallier and Lutton, 1966). When 3 months old, they were divided into 5 groups which were each fed an experimental diet for two months before the isotopes were administered (table 1). The diets differed only in the nature of the lipid offered : lard (U), triolein (Ol), tripalmitin (Pa), tristearin (St) or trierucin (Er). The last group included 18 rats, used and killed in groups of 6 at 4 a.m. (Er_4), noon (Er_{12}) and 8 p.m. (Er_{20}). The results on group U, used as the reference, have been published (Lutton and Chevallier, 1972*b*). The phytosterol diet concentration (mainly β-sitosterol) was very low (0.004 p. 100), and that of the cholesterol was between 0.028 and 0.050 p. 100 (table 2).

TABLE 2

Body weight, food intake, cholesterol levels and fecal sterol elimination of rats fed 5 different experimental diets for 4 months

Diet	U	Ol	Pa	St	Er
Number of rats	8	5	4	4	18 (*d*)
Mean rat weight (g) at the end of the experiment	406 ± 4	522 ± 33 (*b*)	386 ± 11	401 ± 14	334 ± 4 (*a*)
Mean rat weight changes (g/day) during the last month of experiment	0 ± 0.1	+ 0.8 ± 0.1 (*a*)	+ 0.7 ± 0.2 (*a*)	+ 0.6 ± 0.2 (*a*)	— 0.2 ± 0.1
Mean diet intake (g/day/rat) during the last month of experiment	13.3 ± 0.4	16.0 ± 0.4 (*b*)	17.5 ± 0.8 (*c*)	17.6 ± 0.5 (*b*)	13.4 ± 0.2
Average energy intake (kcal/day/rat)	63.8	76.8	84.0	84,5	64.3
Cholesterol concentration (mg/100 g) :					
Diet	28	50	38	40	36
Plasma :					
— free	22 ± 2	25 (*f*)	17 ± 2 (*c*)	18 ± 2	22 ± 4
— esterified	67 ± 4	74 (*f*)	43 ± 2 (*a*)	49 ± 4 (*b*)	32 ± 1 (*a*)
Liver :					
— free	205 ± 6	163 ± 7 (*a*)	165 ± 2 (*a*)	177 ± 4 (*b*)	152 ± 4 (*a*)
— esterified	188 ± 20	171 ± 25	39 ± 2 (*a*)	34 ± 2 (*a*)	73 ± 16 (*b*)
Fecal output (mg/day/rat) :					
Lipids (*e*)	220	238	1 028	1 103	551
Neutral sterols	14.1 ± 0.6	13.0 ± 0.9	12.7 ± 1.6	25.6 ± 1.0 (*a*)	21.8 ± 0.5 (*a*)
DUC (g p. 100)	92.5	92.9	73.3	71.5	81

Mean ± SEM.
(*a*) Significantly different at $P \leqslant 0.001$ vs U.
(*b*) Significantly different at $P \leqslant 0.01$
(*c*) Significantly different at $P \leqslant 0,05$.
(*d*) Three groups of 6 animals killed at 4 a. m., noon and 8 p. m.
(*e*) As estimated from the alcohol extract.
(*f*) Mean (pooled samples).

(*g*) Apparent digestive utilisation coefficient (DUC p. 100) $= \dfrac{\text{Ingested fats (mg/d)} - \text{Fecal fats (mg/d)}}{\text{Ingested fats (mg/d)}}$.

Isotope equilibrium experiments. — The isotopes were administered for 8 weeks (Lutton and Chevallier, 1972*a*) by subcutaneous injections (^{3}H-cholesterol, 0.2 mg, 1-2 μCi/d/rat) and oral way (4-^{14}C-cholesterol). Trace amounts of 4-^{14}C-cholesterol (0.25 to 0.5 μCi/100 g diet) solubilized in ethylether were added to the diet and the ethylether was then evaporated. The principle and validity of the isotope equilibrium method has been discussed previously (Chevallier and Lutton, 1966 ; Lutton and Chevallier, 1972*a*). Feces and urine were collected during the 5th, 6th, 7th and 8th weeks of the experiment and pooled by week. At the end of the 8th week, the rats were killed by intraaortic puncture under nembutal anesthesia.

The liver was removed after washing the cardiovascular system with saline. The heparinized blood was centrifuged (2 200 g at 4 °C for 20 min) and the plasma and the red cells were recovered. The intestinal contents were collected with saline in two groups of rats (Ol and Er). In order to separate the micellar fraction from the sediment (McIntyre *et al.*, 1971), they were centrifuged at 54 000 g for 30 min. After washing with citrate buffer, the intestinal walls were collected and separated into two equal fractions.

The isotopic and chemical methods have been described (Chevallier and Lutton, 1966). Fecal cholesterol was analyzed by thin-layer chromatography (Sulpice *et al.*, 1978). The radiactivities were measured in PPO-dimethyl POPOP toluene solution with a liquid scintillation spectrometer. The rates of cholesterol absorption, internal and external secretions, excretion and transformation into bile acids were calculated (Lutton and Chevallier, 1972*a*) together with the radioactivities of neural fecal sterols and fecal bile acids and the specific activities of mobile cholesterol (mean value of specific cholesterol activities in plasma, red blood cells and liver) and dietary cholesterol. The formulas applied are given in the footnotes of table 3.

Results.

Body weight, food intake, cholesterol levels and fecal sterol elimination (table 2). — Food intake was the same for Er and U rats but higher for Ol, Pa and St animals. Mean body weight increase followed a similar pattern. The cholesterol concentration was significantly lower in the plasma of Pa, St and Er rats. The level of esterified cholesterol in the liver varied ; it was greatly decreased in Pa, St and Er rats. Fecal sterol elimination was practically unaffected when lard was replaced by triolein or tripalmitin, but it was doubled after feeding a tristearin or trierucin diet.

Rates of the processes of fecal elimination and cholesterol turnover (table 3). — Replacement of lard by triolein or tripalmitin did not affect the intestinal absorption coefficient : it was greatly decreased, however, after feeding a tristearin (34 $\pm$ 1 p. 100) or trierucin (45 $\pm$ 1 p. 100) diet. Like fecal excretion, the external cholesterol secretion was doubled in St and Er rats as compared to U, Ol and Pa rats. The daily rate of cholesterol degraded into bile acids was twice as much in Pa or Er rats as in U or Ol animals ; it was not significantly changed in St versus U and Ol rats. Internal secretion and total synthesis rates were practically unchanged when the diets contained triolein instead of lard, whereas those rates were 2 to 3 times higher when tristearin,

tripalmitin or trierucin replaced lard. When the results were calculated on the basis of body weight, the data yielded the same results.

TABLE 3

Absorption coefficient of dietary cholesterol and rates of processes involved in fecal elimination and cholesterol turnover (1)

Diet	U	Ol	Pa	St	Er
Ingestion	3.4 ± 0.1	8.1 ± 0.4	6.7 ± 0.5	7.6 ± 0.2	4.7 ± 0.2
Absorption coefficient (p. 100)	71 ± 3	73 ± 3	75 ± 4	34 ± 1 (a)	45 ± 1 (a)
Absorption (mg/day)	2.4 ± 0.1	5.9 ± 0.3	5.0 ± 0.5	2.4 ± 0.1	2.1 ± 0.1
External secretion (mg/day)	2.9 ± 0.3	2.1 ± 0.4	3.4 ± 0.5	6.0 ± 0.7 (c)	6.0 ± 0.2 (b)
Excretion (mg/day)	4.9 ± 0.2	5.3 ± 0.2	3.8 ± 0.4 (c)	10.5 ± 0.2 (a)	10.3 ± 0.8 (a)
Urinary excretion (mg/day)	0.8 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
Transformation into bile acids (mg/day)	9.2 ± 0.7	13.2 ± 0.9	28.4 ± 5.2 (a)	13.0 ± 0.3	24.8 ± 1.9 (a)
Internal secretion (mg/day)	12.5 ± 0.6	14.4 ± 1.2	28.4 ± 4.6 (b)	22.3 ± 0.7 (a)	33.2 ± 2.8 (a)
Total synthesis (mg/day)	15.4 ± 0.7	16.5 ± 1.3	31.8 ± 4.8 (b)	28.3 ± 0.9 (a)	39.2 ± 3.0 (a)

Mean ± SEM. (a) $P \leqslant 0.001$ vs U ; (b) $P \leqslant 0.01$; (c) $P \leqslant 0.05$ vs U.

(1) Data were calculated as follows (Lutton and Chevallier, 1972) : Absorption coefficient $AC = m_A/m_I \times 100$ with m_A : amount of dietary cholesterol absorbed ; m_I : amount of dietary cholesterol ingested. Absorption $m_A = m_I - m_{NA}$ with m_{NA} amount of unabsorbed dietary cholesterol. Non-absorption: $m_{NA} = R_{NA}/r_A$ with R_{NA} : total radioactivity of unabsorbed ^{14}C-cholesterol ; r_A : specific activity of dietary cholesterol. $R_{NA} = R_{F_1} - R_{FE}$ with R_{F_1} : ^{14}C radioactivity of neutral sterol ; R_{FE} : ^{14}C radioactivity of fecal excretion source. $R_{FE}\ ^{14}C = m_{FE} \times rp_1$ with rp_1 : specific ^{14}C activity of mobile cholesterol ; m_{FE} : fecal excretion rate. $m_{FE} = R_{F_2}/rp_2$ with r_{F_2} : ^3H radioactivity of fecal neutral sterol ; rp_2 : specifical ^3H activity of mobile cholesterol. External secretion : $m_{ES} = m_F - m_{NA} - m_{FE}$ with m_F : total fecal cholesterol. $m_F = R_F/r_F$ with r_F : ^3H or ^{14}C radioactivity of fecal neutral sterol ; r_F, specific ^3H or ^{14}C activity of fecal cholesterol. Transformation into bile acids $= mT = R_{AC}/rp_1$ with R_{AC} : ^{14}C radioactivity of fecal acidic fraction. Internal secretion (two calculations) :

— $m_{IS} = m_T + m_{FE} + m_{UE} + m_{CI} - m_A$ with m_{UE} : urinary excretion rate ; $m_{UE} = R_U/rp_2$ with RU : ^3H radioactivity recovered in urine per day ; m_{CI}: : body cholesterol increase (estimated from body weight increase).

— $m_{IS} = m_A \times RB \times \left(\frac{r_A - rp_1}{rp_1}\right)$ with RB : radioactive balance ; $RB = {}^{14}C_{output}/{}^{14}C_{input}$.

Total synthesis : $m_{SY} = m_{IS} + m_{ES}$.

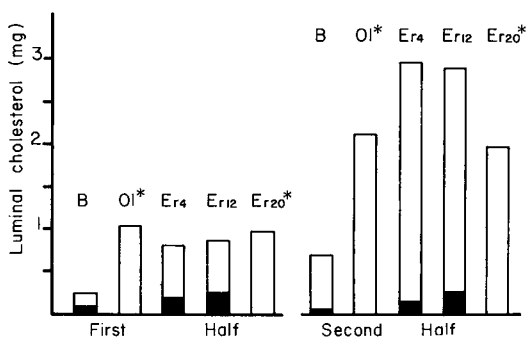

FIG. 1. — *Luminal cholesterol content in the first and second halves of the intestine in rats fed triolein* (Ol) *and trierucin* (Er)*-rich diets.* The results are compared to those of rats receiving a basal normolipid diet (B), containing 0.05 p. 100 of cholesterol. Er_4, Er_{12}, Er_{20} : animals killed at 4 a. m., noon and 8 p. m. The micelles ▬ of groups B, Er_4 and Er_{12} only were separated from the sediment.

Mean amounts of cholesterol in Ol and Er rat intestine. — The luminal cholesterol content increased from the jejunum to the ileum (fig. 1). It was always 4 to 6 times higher in the intestinal contents of Ol and Er rats than in those of rats fed a basal 8 p. 100 diet (Chevallier and Lutton, 1966), containing a similar concentration of cholesterol (0.05 p. 100). This increased quantity of cholesterol in the intestinal contents was seen throughout the day, as shown by the results on the Er rats.

The amounts of dietary cholesterol in the intestine (contents and walls) in Ol and Er rats are shown in table 4. The mean amounts of dietary cholesterol found in the intestinal contents of Er_4 and Er_{12} rats were similar to those of Ol rats. In the walls, however, less was found in Er than in Ol animals.

TABLE 4

Dietary cholesterol distribution (mg) *in the content and the wall of the small intestine.*

		Ol	Er_4	Er_{12}	Er_{20}
Content	first half	0.16	0.09	0.07	0.09
	second half	0.44	0.50	0.46	0.26
	sum	0.60 (8)	0.59 (12)	0.53 (11)	0.37 (7)
Wall	first half	0.97 ± 0.04	0.54 ± 0.04	0.40 ± 0.05	0.43 ± 0.04
	second half	1.05 ± 0.04	0.40 ± 0.04	0.30 ± 0.04	0.65 ± 0.05
	sum	2.02 ± 0.05 (28 ± 1)	0.94 ± 0.05 (19 ± 1)	0.70 ± 0.06 (15 ± 2)	1.08 ± 0.07 (21 ± 2)

Mean content or wall values from 5 (Ol) or 6 (Er) animals ± SEM ; in parenthesis : p. 100 of ingested cholesterol. (For the symbols Ol, Er_4, Er_{12}, and Er_{20}, see fig. 1.)

Quantity of dietary cholesterol (mg) in the contents or wall ($Q_{D.C.}$) was obtained as follows :

$$Q_{D.C.} = \frac{R_1 - R_2}{r_A}.$$

R_1 = ^{14}C cholesterol radioactivity in the contents or wall.
R_2 = plasma ^{14}C-cholesterol radioactivity in the contents or wall
r_A = specific ^{14}C-cholesterol activity of dietary cholesterol

$$R_2 = \frac{R_3}{rp_2} \times rp_1$$

R_3 = ^{3}H cholesterol radioactivity in the contents or wall.
rp_2 = specific ^{3}H-cholesterol activity of mobile cholesterol.
rp_1 = specific ^{14}C-cholesterol activity of mobile cholesterol.

Specific cholesterol activities in the intestine and feces of Er rats. — The specific ^{3}H-cholesterol activities in the intestine (cells and contents) and the feces of rats fed a diet with 20 p. 100 of trierucin, expressed as a percentage of mobile specific ^{3}H-cholesterol activity, are shown in table 5. The values of specific endogenous fecal cholesterol activity, estimated from the specific activity of total fecal cholesterol and the mass of total fecal cholesterol and unabsorbed dietary cholesterol, are also given.

TABLE 5

Specific ^3H activities in intestinal cells and contents and in feces of rats fed a 20 p. 100 trierucin diet

		Er_4	Er_{12}	Er_{20}
Cells	First half	65 ± 3	56 ± 3	68 ± 4
	Second half	64 ± 3	60 ± 4	59 ± 5
Contents	First half	81	69	70
	Second half	76	68	70
Feces	Total cholesterol	62 ± 3	51 ± 4	57 ± 4
	Endogenous cholesterol (a)	72 ± 3	58 ± 2	66 ± 2

The intestine was divided into two halves. Results are expressed as a percentage of mobile specific ^3H-cholesterol activity. At isotope equilibrium, specific ^3H-cholesterol activity in bile equals mobile specific ^3H-cholesterol activity (mean values ± SEM for cells and feces ; mean for contents).

(a) The specific activity of endogenous fecal cholesterol (SA_E) is calculated as follows :

$$SA_E = r_F \times \frac{m_F}{m_F - m_{NA}}$$

with : r_F = specific ^3H activity of total fecal cholesterol,
m_F = total fecal cholesterol,
m_{NA} = unabsorbed dietary cholesterol.

Discussion.

The nature of the dietary fatty acids modifies the numerous processes involved in the turnover and the plasma concentration of cholesterol. Although reports in the literature are conflicting as regards the effect of saturated versus unsaturated dietary fats on plasma cholesterol concentration, the saturated fats are generally considered to raise the plasma cholesterol level, while the polyunsaturated ones are thought to decrease it (Reiser *et al.*, 1963). In the present study, we measured all the cholesterol turnover processes in rats fed a lipid-rich, semi-purified diet for 4 months in the form of 20 p. 100 of triglycerides, containing one long-chain fatty acid. Under these conditions, feeding a tripalmitin, tristearin or trierucin diet decreased cholesterolemia, while triolein did not change it. We previously reported an inverse relationship between the concentration of plasma cholesterol and the rate of internal secretion (Chevallier, Mathé and Lutton, 1976) ; the present results agree with that observation. Moreover, the concentration of plasma esterified cholesterol was significantly lower in Er, Pa and St rats than in U or Ol animals. The rat incorporates a very small amount of erucic acid into plasma phospholipids and cholesterol esters (Carroll, 1962). This suggests that the behaviour of erucic acid towards LCAT * may be similar to that of saturated fatty acids and not to that of unsaturated ones. The concentration of esterified cholesterol in the liver also decreased significantly in Er, Pa and St rats as compared to those of groups Ol and U. In a previous study, we demonstrated a relationship between the absorption coefficient of dietary cholesterol and the rates of some of its

* Lecithin cholesterol acyltransferase.

turnover processes (Chevallier and Lutton, 1973 ; Lutton and Chevallier, 1966). Thus, we shall discuss first the variations of the absorption coefficient.

Cholesterol absorption. — The absorption coefficient of dietary cholesterol was high for U, Ol and Pa rats, but decreased for the Er group and was even lower for St rats. Thus, for the four groups of rats (U, Ol, St and Er), the DUC * of fats and cholesterol absorption varied similarly. This observation is possible since cholesterol and fatty acids (mainly in the form of monoglycerides) from the same carrier particules, namely the micelles, enter the intestinal epithelial cells (Hofmann and Borgström, 1962). The low DUC of fats observed with Pa rats is probably due to the very low DUC of the two palmitate molecules resulting from tripalmitin hydrolysis, while the DUC of glycerol 2 palmitate is higher (Thieulin, 1968).

To determine the factors modifying intestinal absorption of cholesterol, we studied the concentration and origin of that molecule at different levels of the small intestine in rats whose cholesterol absorption was high (Ol) or low (Er). These results can also be compared to those obtained in rats receiving a normolipid (8 p. 100) diet (B) (fig. 1) containing the same cholesterol concentration. The quantity of cholesterol in the intestinal contents of rats fed a triolein or trierucin-rich diet was 4 to 5 times higher at all the intestinal sites studied than that of rats eating a normolipid diet (fig. 1). Moreover, the results of Er rats show that that increase occurred throughout the day. The quantity of micellar cholesterol in the contents was also higher in the Er rats than in those fed the normolipid diet. However, the micellar cholesterol only corresponded to 25-29 and 4-8 p. 100 of the cholesterol present in the contents of the jejunum and the ileum, respectively, in Er rats, while it attained 46 and 11 p. 100 in B rats. Although it was increased, micelle formation was proportionally lower in Er rats than in the normolipid controls (B).

For the same mass of ingested cholesterol, the quantity of dietary cholesterol found in the intestinal contents was quite similar in Ol and Er rats (table 4), but more dietary cholesterol was found in the walls of Ol rats (2.02 ± 0.05 mg, i.e. 28 ± 1 p. 100 of ingested cholesterol) than in those of Er rats (0.7 to 1.08 mg, depending on the time killed, i.e. 18 ± 3 p. 100 of ingested cholesterol). It is noteworthy that the percentages of ingested cholesterol present in the intestinal walls in Ol and Er rats (28 and 18, respectively) are in the same ratio as their absorption coefficients (73 and 45). In Ol rats whose intestine was divided into four equal fractions (results not tabulated), most of the absorbed dietary cholesterol (71 p. 100) was present in the second and third quarters of the wall (33 and 38 p. 100, respectively). This suggests that dietary cholesterol absorption takes place in the second, as well as in the first, half of the small intestine. The results obtained with Er rats lead to the same conclusions and agree with previous data obtained in rats fed a basal normolipid diet (Lutton and Brot-Laroche, 1979). The present data (fig. 1) show that quantities, like the proportions of micellar cholesterol, decrease in the intestinal contents from the jejunum to the ileum as reported by McIntyre *et al.* (1971). Also, the intestinal micellar cholesterol and the quantity of absorbed cholesterol in the contiguous wall cannot be strictly correlated as is generally believed (McIntyre *et al.*, 1971). An important factor, not taken

* Digestive utilization coefficient.

into account, is the time of the presence of the compound at a given level. In a previous study, we showed that the transit time for cholesterol is enhanced towards the caecal valvulae (Chevallier and Lutton, 1972). The fact that that time increases while micelle formation decreases partly explains why cholesterol absorption is greater in the ileum than micelle data might suggest.

In the intestinal contents, endogenous cholesterol (obtained by subtracting dietary from total cholesterol) was 3-4 times higher in Ol and Er rats than in the normolipid controls. The fact that those rats ingested daily a similar quantity of dietary cholesterol, would suggest that the rate of endogenous cholesterol arriving in the intestinal lumen was stimulated by a fat-rich diet. On the other hand, dietary cholesterol was more diluted in rats fed a hyperlipid diet than in those fed a normal one. Endogenous cholesterol is supplied by the bile and the intestinal mucosae. In the rat fed a basal diet, the biliary cholesterol rate (2-3 mg/d) was 4 to 5 times less than that of the mucosae. Moreover, biliary cholesterol comes mainly from the plasma, while mucosal cholesterol is supplied by the plasma and cholesterol synthesis in approximately similar proportions (Chevallier and Rodrigues-Branco, 1963 ; Chevallier and Lutton, 1972). In rats fed a hyperlipid diet, the contribution of biliary cholesterol to endogenous cholesterol in the lumen is probably increased since Boquillon and Clément observed that bile cholesterol elimination was strongly enhanced after feeding rats diets containing 20 p. 100 of corn oil (Boquillon and Clément, 1979). But mucosae still remain the main source of endogenous cholesterol since at isotope equilibrium, the specific activity of endogenous ^{3}H-cholesterol in Er rat feces was more similar to that of cholesterol in the intestinal cells than to that of biliary cholesterol (table 5). If biliary and intestinal cell cholesterol is homogeneously mixed in the lumen, we estimate from the above data that the daily rate of endogenous cholesterol poured into the lumen by the mucosae is 2.5 times higher than that of biliary cholesterol. However, this calculation is known to be approximate because the behaviour of biliary cholesterol is not absolutely identical to that of mucosal cholesterol (Lutton and Brot-Laroche, 1979), and because most of the biliary cholesterol is reabsorbed. Consequently, the origins of endogenous cholesterol eliminated in the feces (fecal excretion plus fecal external secretion) are mainly mucosal.

Fecal excretion and fecal external secretion of cholesterol. — It should be noted first that the term « endogenous sterol excretion », generally used in the literature, corresponds to our sum of « fecal excretion plus fecal external secretion » since the cholesterol of these two origins may be distinguished with the isotope equilibrium method (Chevallier, 1967 ; Lutton, 1976). The results in the literature are conflicting, but most studies in man (Moore *et al.*, 1968 ; Connor *et al.*, 1969 ; Nestel, Havenstein and Whyte, 1973 ; Nestel *et al.*, 1975) and in rat (Danielsson and Tchen, 1968) indicate that « endogenous sterol excretion » is higher with diets rich in unsaturated fatty acids. However, this rise is often temporary and the rate of « endogenous sterol excretion » returns to normal when a new dynamic equilibrium is reached (Nestel *et al.*, 1975).

In our isotope equilibrium experiments, the dynamic equilibrium is well established since the adult rats were fed the diets for 4 months. Under these conditions, we observed that the rates of fecal excretion and fecal external secretion were enhanced

after feeding tristearin or trierucin-rich diets (i.e. rats having a low cholesterol absorption coefficient), and were unchanged after feeding triolein or tripalmitin-rich diets (cholesterol absorption coefficient unchanged). These data agree with the previous relationships drawn from 30 experiments (Chevallier and Lutton, 1973 ; Lutton and Chevallier, 1976), correlating fecal excretion (m_{FE}) or fecal external secretion (m_{ES}) to the absorption coefficient (AC) of dietary cholesterol

$$m_{FE}\ (mg/d) = -0.136\ AC\ (p.\ 100) + 14.99\ (n = 29\ ;\ r = 0.92\ ;\ P \leqslant 0.001) \quad (1)$$
$$m_{ES}\ (mg/d) = -0.056\ AC\ (p.\ 100) + 6.96\ (n = 26\ ;\ r = 0.84\ ;\ P \leqslant 0.001). \quad (2)$$

Cholesterol synthesis. — From the previous experiment cited above (Lutton and Chevallier, 1976), a relationship between cholesterol synthesis (m_{SY}) and the absorption coefficient was established (25 groups of rats out of 30 assessed as « typical » rats).

$$m_{SY}\ (mg/d) = -0.232\ AC\ (p.\ 100) + 35.05\ (n = 25\ ;\ r = 0.81\ ;\ P \leqslant 0.001). \quad (3)$$

These « typical » rats had the following characteristics ;

1. The intestine was the major source for internal cholesterol secretion.
2. The rates of fecal excretion, fecal external secretion and total synthesis were inversely correlated to the absorption coefficient (see above relationships). « Atypical » rats were characterized either by an high extra-digestive internal secretion (mammary glands or placenta during lactation or pregnancy, bile duct in liver ligated or cholestyramine feeding) or a modified partition between internal and external cholesterol secretions.

If we analyse the present results from this viewpoint, Ol and St rats can be assessed as « typical » rats. In Er and Pa animals, on the contrary, cholesterol synthesis is higher than what can be expected from the relationship (3). The increase in cholesterol synthesis in the last two groups consequently seems to be due to strong extra-digestive synthesis, as has been observed previously after 2 p. 100 cholestyramine (Lutton, Mathé and Chevallier, 1973) or a commercial chow rich in fiber (Mathé *et al.*, 1977). In rats fed a 2 p. 100 cholestyramine-supplemented diet, cholesterogenesis can be increased tenfold in the liver. Such increased hepatic cholesterogenesis has been noted by Carroll (1959) after erucic acid or trierucin ingestion and by Reiser *et al.* (1963) after triplamitin intake, while stearic acids seems to be ineffective (Carroll, 1959).

Transformation into bile acids. — This study shows little change in the rate of cholesterol transformation into bile acids in Ol and St as compared to U rats. On the contrary, these rates were doubled in the rats fed the tripalmitin and trierucin diets. In the dynamic equilibrium of cholesterol in the rat, is the rate of cholesterol transformation into bile acids directly regulated by a feedback mechanism or is it modulated by the quantity of cholesterol entering the organism (absorption plus synthesis) ? In previous studies (Lutton, Mathé and Chevallier, 1973 ; Mathé *et al.*, 1977), we have provided some evidence in favour of the latter hypothesis. The results of the present study also point to the latter hypothesis. The increase of internal secretion in St, Pa and Er rats is mainly compensated for by elevated fecal excretion (St) rather than by enhancement of the bile acid output (Pa) or a stimulation of both the parame-

ters (Er). In a recent study, Björkheim *et al.* (1978) showed that the rate of 7α-cholesterol hydroxylation is faster after feeding a 20 p. 100 trierucin or tripalmitin diet than after a 20 p. 100 triolein diet. Our results are similar to theirs, if we admit that the rate of 7α-cholesterol hydroxylation reflects that of bile acid synthesis. One hypothesis advanced by Björkheim *et al.* to explain the stimulation of 7α-hydroxylation by tripalmitin or trierucin consisted in correlating bile acid and fatty acid absorptions. If we adapt this interpretation to the Er group (low cholesterol absorption coefficient), trierucin should also stimulate 7α-hydroxylation. On the contrary, the results of the present study suggest that 7α-hydroxylation is not modified in Er as compared to Ol rats (table 3). Another explanation could be that the presence of certain fatty acids increases the pool and the number of bile acid recirculations, inhibiting 7α-hydroxylase by a feedback mechanism. Boquillon and Clément observed an higher elimination of bile acids in the bile of rats fed a 20 p. 100 lipid diet (mutton tallow or corn oil) as compared to rats fed a 7 p. 100 lipid diet or even a 20 p. 100 lard diet. As isotope equilibrium, the ratio, ^{14}C radioactivity in the saponifiable fraction of intestinal contents plasma specific ^{14}C-cholesterol activity, gives the quantity of bile acids present in the intestinal contents at killing. The mean bile acid content in the intestines of Ol, Er_4, Er_{12} and Er_{20} rats was 42, 88, 78 and 47 μmoles, respectively. Although these values differed greatly during the day, might the possible increase in the bile acid pool due to trierucin be the consequence of modified bile acid reabsorption, or might it be related to an increased intestinal transit time, as recently suggested by Riotto *et al.* (1980) ? Further studies are needed to clarify these points.

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Résumé. Des rats mâles adultes ingèrent pendant 4 mois un régime semi-synthétique contenant 20 p. 100 de saindoux ou de triglycérides homogènes (tripalmitine, tristéarine, trioléine ou triérucine). Les vitesses des processus du renouvellement du cholestérol mobile (absorption, synthèse, dégradation en acides biliaires, excrétions fécale et urinaire) sont mesurées par une méthode d'équilibre isotopique.

Le coefficient d'absorption du cholestérol, égal à 71 ± 3 p. 100 chez les rats ingérant le régime à base de saindoux est inchangé chez ceux ingérant la trioléine (73 ± 3 p. 100) ou la tripalmitine (75 ± 4 p.100) et fortement abaissé chez les rats recevant la triérucine (45 ± 1 p. 100) ou la tristéarine (34 ± 1 p. 100). Certains aspects topologiques et fonctionnels de l'absorption du cholestérol sont précisés. La masse de cholestérol endogène présente dans le contenu intestinal est plus grande chez le rat ingérant un régime hyperlipidique que chez celui recevant un régime normolipidique. Il en est de même du cholestérol présent dans la fraction micellaire mais l'augmentation de ce dernier est moindre que celle du cholestérol endogène. Il n'existe pas de relation directe entre la quantité de cholestérol dans les micelles du contenu intestinal et la masse de cholestérol absorbé, l'absorption du cholestérol alimentaire ayant lieu principalement au niveau des deuxième et troisième quarts de l'intestin tandis que la fraction du cholestérol du contenu sous forme micellaire décroît du jéjunum vers l'iléum.

L'excrétion et la sécrétion externe fécales de cholestérol varient de façon inversement proportionnelle au coefficient d'absorption du cholestérol alimentaire, ainsi qu'il a été décrit précédemment. La sécrétion interne de cholestérol est stimulée de 2 à 3 fois chez les rats ingérant la tristéarine (22,3 ± 0,7 mg/j), la tripalmitine (28,4 ± 4,6 mg/j) et la triérucine (33,2 ± 2,8 mg/j) comparés à ceux ingérant le régime à base de saindoux (12,5 ± 0,6 mg/j) ou de trioléine (14,4 ± 1,2 mg/j). L'ingestion de tripalmitine et de triérucine induit une forte stimulation de la cholestérogenèse extradigestive (probablement celle du foie) tandis que celle due à la tristéarine ne paraît pas être d'origine hépatique. Les

variations de l'élimination quotidienne des acides biliaires peuvent être interprétées comme celle d'un paramètre adaptatif modulant sa vitesse en fonction de l'apport (absorption plus synthèse) de cholestérol.

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